

## Chromosomal Characteristics of *Oxygyne shinzatoi* (Burmanniaceae), and its Phylogenetic Significance

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The chromosome characteristics of *Oxygyne shinzatoi*, subtribe *Oxygyninae*, tribe *Thismiaeae*, Burmanniaceae, are described and the phylogenetic significance of this species is discussed. The chromosome number of *O. shinzatoi* is  $2n = 18$ , and the basic chromosome number is thought to be  $x = 9$ . This differs from the  $x = 6$  of *Thismia*, subtribe *Thisminae*, tribe *Thismiaeae*, and the  $x = 6$  or  $8$  of *Burmannia*, tribe *Burmanniaeae*. The sizes of *O. shinzatoi* and of *T. abei* chromosomes differ from those of *Burmannia*. The interphase nucleus of *O. shinzatoi* is of the complex chromocenter type, in contrast to that of *T. abei*. This result suggests that there are large differences karyomorphologically between the subtribes *Thisminae* and *Oxygyninae*.

Key words: basic chromosome number, Burmanniaceae, chromosome, karyotype, *Oxygyne shinzatoi*, phylogeny

The Burmanniaceae are a unique family that contains many achlorophyllous, mycoheterotrophic genera and species. Because of the difficulty in obtaining material for detailed morphological, anatomical, taxonomic, and other analyses, the phylogenetic relationships within the family are still unclear. Jonker (1938) classified the family into two tribes, *Burmanniaeae* Miers. and *Thismiaeae* Miers., and further divided the former into the subtribes *Burmanninae* Benth. & Hook. and *Apterinae* Miers. and the latter into the subtribes *Thisminaee* Miers. and *Oxygyninae* Jonk. Rübsamen (1986), Maas *et al.* (1986) and Maas-van de Kamer (1998) followed almost the same arrangement. Recent molecular phylogenetic studies have revealed that the tribes *Burmanniaeae* and *Thismiaeae* are only distantly related (Neyland 2002, Neyland & Hennigan

2003, Merckx *et al.* 2006), and some authors (*e.g.*, Caddick *et al.* 2002, Neyland 2002, Woodward *et al.* 2007) have treated these tribes as distinct families. However, the genus *Oxygyne* Schltr., subtribe *Oxygyninae*, tribe *Thismiaeae* has not yet been analyzed, although several genera have been analyzed, such as *Aptenia* Nutt., *Burmannia* L., *Cymbocarpa* Miers., *Dictyostega* Miers., *Gymnosiphon* Bl., and *Hexapterella* Urb. from the tribe *Burmanniaeae*, and *Afrothismia* Schltr., *Haplothismia* Airy Shaw, and *Thismia* Griff. from the tribe *Thismiaeae*. To understand the phylogenetic relationships of the members of the Burmanniaceae and related groups, further detailed analyses using multidisciplinary approaches for all of the Burmanniaceae genera are required.

Karyologically, members of this family show a range of basic chromosome numbers: the genus

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*Burmannia* has 8 ( $2n = 32$  to 136; Larsen 1963), the genus *Gymnosiphon* has 6 to 8 or 14 to 17 (Rübsamen 1986), and the genus *Thismia* has 6 (Aoyama *et al.* 1978) or 11-14 (Rübsamen 1986). To better understand the phylogenetic relationships between the genera in this family, data on the karyological characteristics of more genera and species are required. However, most species in this family are difficult to find, and little living material has been available for analyses such as karyotyping. For example, we found and described a new species of the genus *Thismia*, *T. mullerensis* H. Tsukaya et H. Okada from Borneo, but only one specimen has been reported so far (Tsukaya & Okada 2005) and no karyotype information is available. Twenty out of the 31 species of the tribe *Thismiaeae* listed in Jonker (1938) have only been collected once. Similarly, the genus *Oxygyne*, which also belongs to this family, was established from one species, *O. triandra* Schltr., which was reported from Cameroon, West Africa (Schlechter 1906), but the type locality of *O. triandra* has been lost and no additional specimen is available (Dr. Thassilo Franke, personal communication). The genus *Oxygyne* is interesting because of its isolated distribution pattern and the large geographical gap between West Africa and Japan. Thus, a detailed analysis of this genus will be important in understanding the geobotany and phylogeny of the Burmanniaceae. The two other remaining species of this genus, *O. shinzatoi* (Hatus.) C. Abe & Akasawa (= *Saionia shinzatoi* Hatus.) and *O. hyodoi* C. Abe & Akasawa, were described from a locality on Okinawa Island, the Ryukyus, Japan (Hatusima 1976, Abe & Akasawa 1989) and in Shikoku, Japan (Abe & Akasawa 1989), respectively. Unfortunately, neither of the two Japanese *Oxygyne* species has been collected recently. Thus, karyotype analysis of *Oxygyne* species has not been possible until now. However, in fall 2006, we collected fresh *O. shinzatoi* material from the type locality and obtained clear images of the karyotype. Here, we report the first description and the uniqueness of the karyo-

type of *O. shinzatoi*.

## Materials and Methods

The material analyzed in this study, *Oxygyne shinzatoi*, was collected from the type locality of the species (Fig. 1): Yona Station, Subtropical Field Science Center, University of the Ryukyus, Kunigami-son, Okinawa Pref., Japan. Voucher specimens are kept in TI (*H. Tsukaya 061008*) and RYU (*M. Yokota s. n.*).

Jonker's (1938) taxonomic treatments, *i.e.*, tribe and subtribe in the family, were employed in this study.

Fresh shoot apices and young flower buds were pretreated with 2 mM 8-hydroxyquinoline aqueous solution for approximately 10 h at about 5°C. They were then fixed in a solution of 2:1:1 EtOH : glacial acetic acid : chloroform for at least one day at about 5°C. The chromosome observation procedures were carried out as described previously (Okada 1984a). The chromosome complement was visualized by aligning the chromosomes (Fig. 2C).

## Results

In the present study, we first determined that the chromosome number was  $2n = 18$  in three *Oxygyne shinzatoi* plants (Fig. 2B). This is the first report of the chromosome number of both this species and of *Oxygyne*. At interphase, nuclear chromatin formed condensed bodies (Fig. 2A) that were darkly stained and irregularly shaped. In some cells, these condensed bodies were aggregated into larger bodies. Diffuse chromatin stained lightly and was fibrous. These karyological characteristics correspond to those of the complex chromocenter type (cf. Tanaka 1971). The chromosome sizes at somatic metaphase varied, ranging from about 2.1 to 4.0  $\mu\text{m}$  (Fig. 2B), and the chromosome sizes gradually decreased (Fig. 2C). Based on detailed observa-

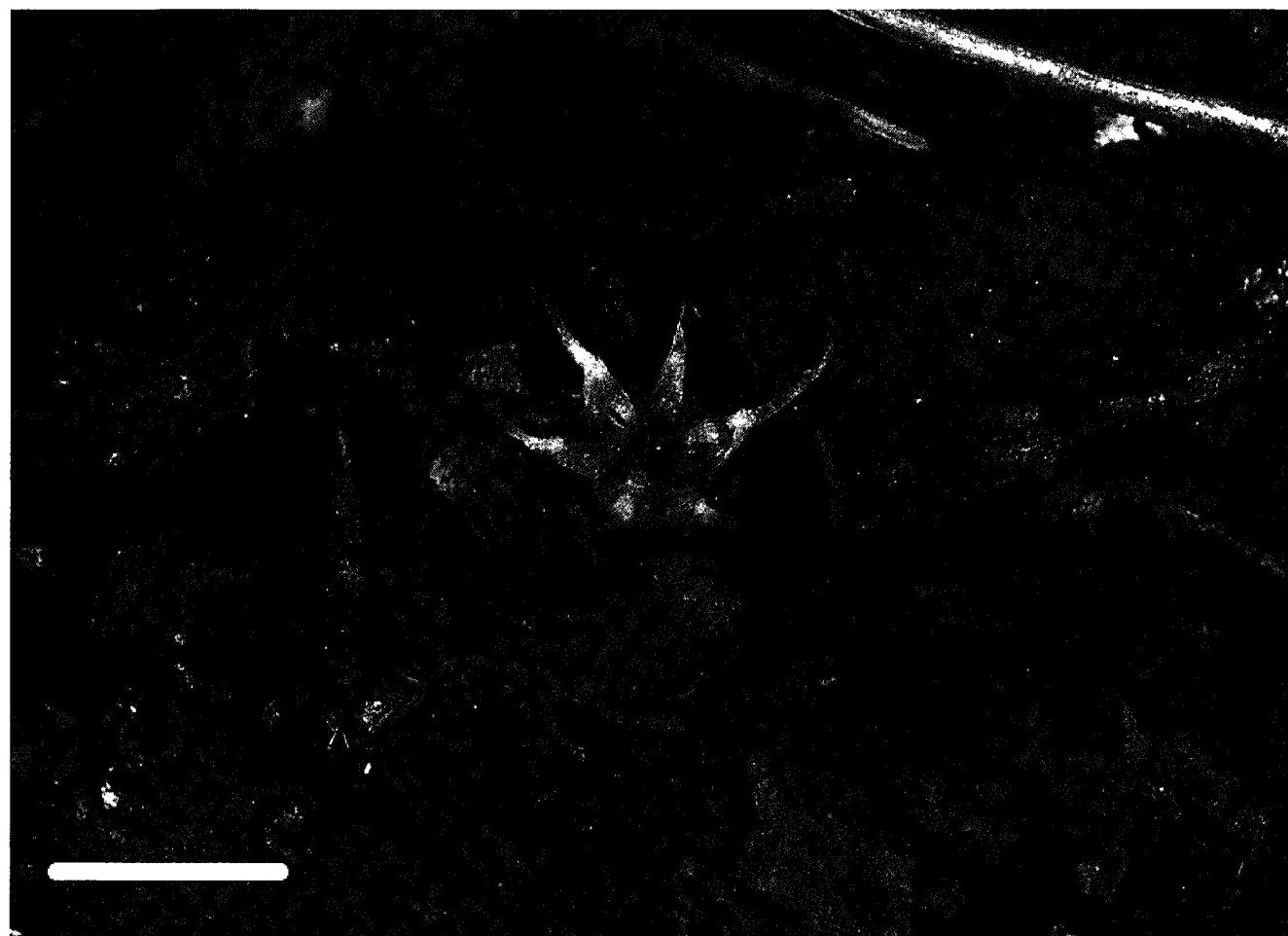


FIG. 1. Flowering individual of *Oxygyne shinzatoi* at the type locality. This species was re-discovered in 2004 from this locality, but has not been found elsewhere from the type locality. Bar = 5 mm.

tions of the chromosome shapes at metaphase (Fig. 2C), we identified nine homologous chromosome pairs, as in the normal diploid species. The first pair was composed of two remarkable metacentric chromosomes.

## Discussion

There is little information on the cytology of the family Burmanniaceae (Table 1). According to Jonker's (1938) classification, the tribe *Burmannieae* contains the subtribe *Burmanninae* (*Burmannia* and two other genera) and the subtribe *Apterinae* (*Gymnosiphon* and five other genera), whereas the tribe *Thismiaeae* is composed of the subtribe *Thisminaee* (*Glaziocharis*, *Thismia*, and four other

genera) and the subtribe *Oxygyninae* (*Oxygyne*). No karyological information has been reported previously for the subtribe *Oxygyninae*. For species in the tribe *Burmannieae sensu* Jonker (1938), Larsen (1963) reported the chromosome numbers for three species found in Thailand:  $n = 16$  in *Burmannia wallichii* (Miers.) Hook. f.,  $2n = \text{ca. } 32$  in *B. coelestis* Don., and  $2n = \text{ca. } 136$  in *B. disticha* L. He noted that the chromosome observations in *B. disticha* were uncertain. Rübsamen (1986) compiled previous reports on the chromosome numbers of some species of the Burmanniaceae and included his observations of chromosome numbers for two *Gymnosiphon* species: *G. breviflorus* Gleason with  $n = \text{ca. } 6-8$ , and *G. recurvatus* Snelders & Maas with  $n = \text{ca. } 14-17$ . Judging from

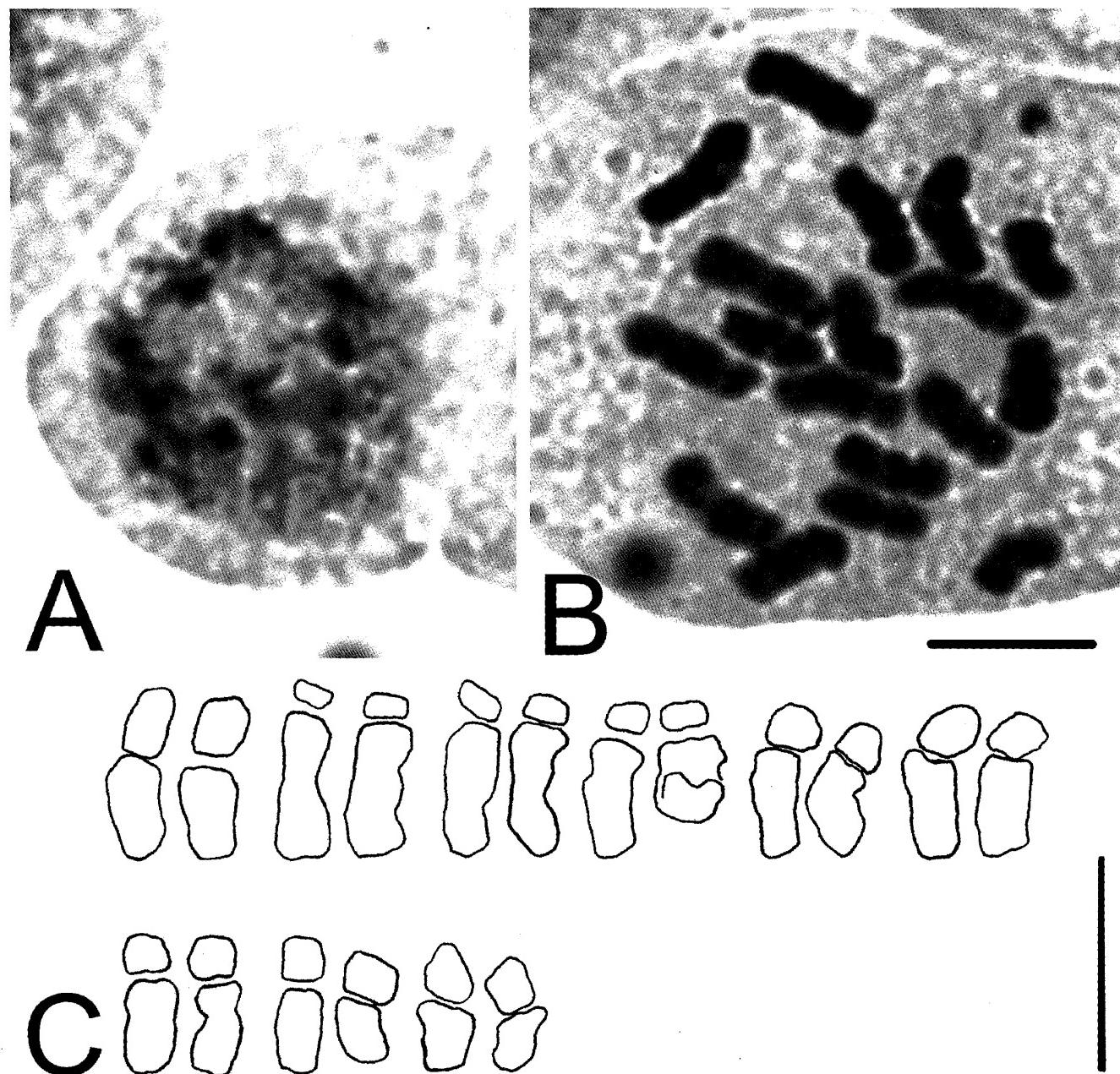


FIG. 2. Somatic chromosomes of *Oxygyne shinzatoi*. A, photomicrograph of interphase nucleus; B, photomicrograph of somatic metaphase chromosomes.  $2n = 18$ . C, Chromosome complement aligned by size. Bars = 5  $\mu\text{m}$ .

the basic chromosome number of the genus *Burmania* (cf. Larsen 1963), the chromosome numbers ( $n$ ) of *G. breviflorus* and *G. recurvatus* are 8 and 16, respectively, or some number derived from 8 and 16. On the other hand, chromosome numbers of  $n = 6$  and  $2n = 12$  were reported for *B. championii* Mart. and *B. lutescens* Becc., respectively (Table 1; Ernst & Bernard 1912a, Schoch 1920). Based on these previous reports, Rübsamen

(1986) claimed that the basic chromosome number of the family was  $x = 6$  or 8.

For the tribe *Thismiaeae sensu* Jonker (1938), Aoyama *et al.* (1978) reported a chromosome number of  $2n = 12$  for *T. abei* (Akasawa) Hatus. (= *Glaziocharis abei* Akasawa), based on clear observations, and Rübsamen (1986) found the chromosome number of *T. luetzelburgii* Goebel & Suss. to be  $n = \text{ca. } 11\text{-}14$  and that of *T. panamensis* F. Muell.

TABLE 1. Available chromosome numbers ( $n$ ,  $2n$ ), basic chromosome numbers, and chromosome sizes of the Burmanniaceae. Taxonomic arrangements followed Jonker (1938).

Species	Chromosome number			Chromosome size	Author(s)		
	$n$	$2n$	$x$				
<i>tribe Burmannieae</i>							
<i>subtribe Burmanniniae</i>							
<i>Burmannia capitata</i> Mart.		ca. 136	?		Schinini 1975 <sup>4)</sup>		
<i>B. championii</i> Thwaites	6	12	6		Ernst & Bernard 1912a <sup>4)</sup>		
	32-36	64-72	8 or 9?		Schoch 1920 <sup>4)</sup>		
<i>B. coelestis</i> D. Don		30-36	?		Ernst & Bernard 1912b <sup>4)</sup>		
	32-36		8 or 9?		Schoch 1920 <sup>4)</sup>		
		ca. 32	8	ca. 2 $\mu\text{m}$ <sup>1)</sup>	Larsen 1963		
		ca. 16	8		Sarkar <i>et al.</i> 1973 <sup>4)</sup>		
<i>B. dasyantha</i> Mart.	ca. 87-99	8?	small <sup>2)</sup>		Rübsamen 1986		
<i>B. disticha</i> L.	20-22		?		Schoch 1920 <sup>4)</sup>		
		ca. 136	?	ca. 1 $\mu\text{m}$ <sup>1)</sup>	Larsen 1963		
<i>B. lutescens</i> Becc.	6	12	6		Ernst & Bernard 1912a <sup>4)</sup>		
		12	6		Schoch 1920 <sup>4)</sup>		
<i>B. stuebelii</i> Hieron. & Schltr.	(14)-16		8		Spitmann 1975 <sup>4)</sup>		
<i>B. wallichii</i> (Miers) Hook.f.	16		8	ca. 1 $\mu\text{m}$ <sup>1)</sup>	Larsen 1963		
<i>subtribe Apterinae</i>							
<i>Gymnosiphon breviflorus</i> Gleason	6-8		8?		Rübsamen 1986		
<i>G. recurvatus</i> Snelders & Maas	14-17		8?		Rübsamen 1986		
<i>tribe Thismieae</i>							
<i>subtribe Thisminiae</i>							
<i>Thismia abei</i> (Akasawa) Hatus. (= <i>Glaziocharis abei</i> Akasawa)		12	6	3.5-1 $\mu\text{m}$ <sup>3)</sup>	Aoyama <i>et al.</i> 1978		
<i>T. javanica</i> F. Muell.	6-8	12	6		Meyer 1909 <sup>4)</sup>		
<i>T. luetzelburgii</i> Goebel & Suss.	11-14		6?	long <sup>2)</sup>	Rübsamen 1986		
<i>T. panamensis</i> (Standley) Jonker	ca. 11-13		6?	long <sup>2)</sup>	Rübsamen 1986		
<i>subtribe Oxygyninae</i>							
<i>Oxygyne shinzatoi</i> (Hatus.) C. Abe & Akasawa		18	9	4.0 - 2.1 $\mu\text{m}$	Present study		

<sup>1)</sup> Calculated from Larsen's (1963) drawings. <sup>2)</sup> Description in Rübsamen (1986). <sup>3)</sup> Description in Aoyama *et al.* (1978).

<sup>4)</sup> Cited from Rübsamen (1986).

to be  $n = \text{ca. } 11-13$ . We believe that the basic chromosome number for the subtribe *Thisminiae* is  $x = 6$ , because the chromosome number of *T. abei* is beyond doubt and the chromosome numbers of both *Thismia* species seem to be derived from those with a ploidy level of  $x = 6$ . However, we found that the chromosome number for *Oxygyne shinzatoi* is  $2n$

= 18 (Fig. 2). Karyotype analysis showed nine pairs of homologous chromosomes, including one pair of remarkable metacentric chromosomes (the first pair in Fig. 2C), suggesting that this species is diploid. Hence, the basic chromosome number for the subtribe *Oxygyninae* is  $x = 9$ . The basic chromosome number for tribe *Thismieae* appears to be  $x = 6$  and

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9. At present, the relationships of the basic chromosome numbers within the family Burmanniaceae,  $x = 6, 8$ , and  $9$ , have not been clarified.

Interestingly, the chromosome sizes of the two tribes differed from each other (Table 1): *Burmannia coelestis*, *B. disticha*, and *B. wallichii* of the tribe *Burmannieae* possessed small chromosomes of only about 1-2  $\mu\text{m}$  (cf. Larsen, 1963), whereas species of the tribe *Thismiaeae* had moderate-sized chromosomes, such as *Thismia abei* with chromosomes of 1-3.5  $\mu\text{m}$  (Aoyama *et al.* 1978) and *Oxygyne shinzatoi* with chromosomes of 2.1-4.0  $\mu\text{m}$ . Rübsamen (1986) noted the contrast in chromosome sizes and shapes within the family: small round chromosomes in *Apteria aphylla* Small, *Dictyostega orobanchoides* Miers, *Gymnosiphon suaveolens* Urb., and *B. dasyantha* Mart., of the tribe *Burmannieae*, and long rod-shaped chromosomes in *T. luetzelburgii* and *T. panamensis* in the tribe *Thismiaeae* (no photographs or drawings were provided). We clarified differences in the morphologies of interphase nuclei in *T. abei* and *O. shinzatoi*. The former were of the rod prochromosome type (Aoyama *et al.* 1978), and the latter were of the complex chromocenter type (cf. Tanaka 1971). Since karyological studies of the Orchidaceae revealed that such differences in interphase nuclei can only be seen in distantly related taxa (Tanaka 1971), the relationships between the tribes *Burmannieae* and *Thismiaeae* and also between the two subtribes *Thisminae* and *Oxygyninae* are likely very distant. Merckx *et al.* (2006) reported that the tribe *Burmannieae* is distantly related to the tribe *Thismiaeae*, based on sequences of the nuclear 18S rDNA and the mitochondrial *nadl b-c* intron. At the same time they show the tribe *Thismiaeae* and *Tacca*, Taccaceae form the same clade. At the present, we can not discuss the karyological relationships between tribe *Thismiaeae* and the genus *Tacca*, because the chromosome information of the genus *Tacca* is still scanty ( $2n = 28$ : *T. chowdhuriana* Deb., Sharma 1970, Chatterjee *et al.* 1989.  $2n = 30$ : *T. involucrata* Schum. & Thonn., Baldwin & Speese 1951.  $2n = 36$ : *T. sp.*, Okada 1984b). The phylogenetic position of the genus *Oxygyne* inferred from molecular data has not yet been examined. Our karyological data strongly suggest the need for further molecular phylogenetic studies of this family, as they revealed large differences between the subtribes *Thisminae* and *Oxygyninae*, and molecular phylogenetic analysis of *O. shinzatoi* is now underway. The present study has established a good basis for further analyses of the unique genus *Oxygyne*.

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